PATENT
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Applicants(s)	:	Lamb et al.
U.S. Serial No.	:	09/310,685
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Art Unit	:	1644
For	:	**NOTCH**

745 Fifth Avenue
New York, NY 10151

VIA HAND DELIVERY

DECLARATION UNDER 37 C.F.R. 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Jonathan R. Lamb, declare and state that:

1. A copy of my *curriculum vitae* demonstrating my education, training and experience is appended hereto. I am a co-inventor for U.S. application Serial No. 09/310,685, and am familiar with the application and its prosecution history. Accordingly, I am considered by my peers to be an expert in the field to which the application pertains, and am otherwise qualified to speak and render expert opinions as to the present application, invention, and issues of the Office Action dated February 26, 2002. Thus, this Declaration is in response to the Office Action.

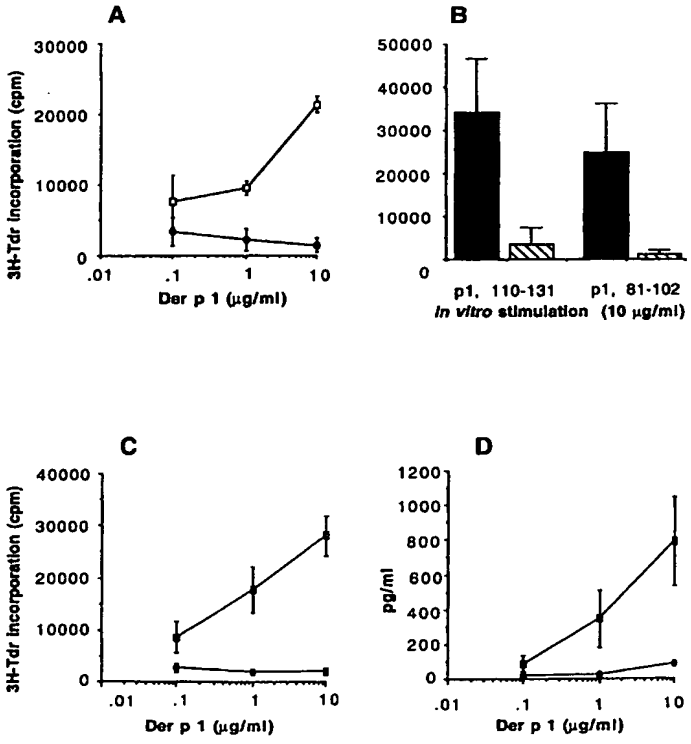
2. The following experiments were performed by me or under my direction, supervision or control, and in the ordinary course of business.

Study 1. Notch ligand expressing antigen-presenting cells inhibit T-cell immunity to the antigen presented.

(A) Mouse antigen-presenting cells (APC) were infected with Notch ligand gene *Serrate1* (•) or control (□) virus, pulsed with dust mite antigen p1, 110-131 peptide and injected into naïve C57BL/6J mice and two weeks later mice were immunized with 50 μg House Dust Mite (HDM) antigen Der p 1/Complete Freund's Adjuvant (CFA). Lymph node (LN) cells were cultured *in vitro* with Der p 1 and T-cell proliferation was measured and the results presented as mean c.p.m. ± SD of four mice per group.

(B) LN cells from mice primed as described above [*Serrate1*$^+$ APC (shaded bars) or a control APC (closed bars)] were cultured *in vitro* with the Der p1 peptides p1, 110-131 or p1, 81-102 at 10μg/ml and proliferation measured. The supernatants from these assays were collected at 24 h and assessed for IL-2 production (C), while 48 h supernatants were assessed for the presence of IFN-γ (D).

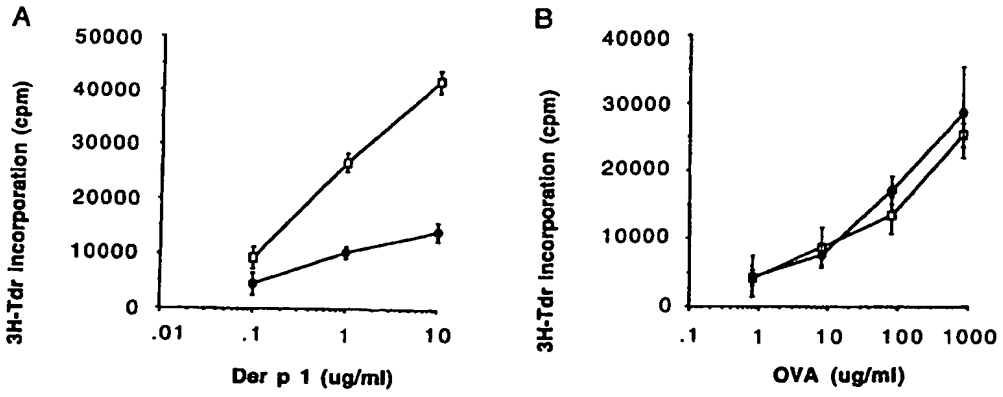
This study shows that antigen-presenting cells expressing Notch ligand are able to inhibit T-cell activity to antigen.



Study 2. Immunization with Notch ligand expressing antigen-presenting cells pulsed with specific peptide induces antigen specific but not global suppression of immunity.

(A) APC were infected with *Serrate1* (•) or control (□) virus pulsed with p1, 110-131 and 2 weeks later the mice were immunized with 50 μg Der p 1/CFA. LN cells were cultured *in vitro* with Der p 1 and proliferation was measured and the results presented as mean c.p.m. ± SD of four mice per group. (B) Mice were injected with *Serrate1*$^+$ APC pulsed with p1, 110-131 as described above but then immunized with OVA/CFA. LN cells were re-stimulated with OVA *in vitro* and proliferation measured as above.

This study shows that antigen-presenting cells expressing Notch ligand in the context of one antigen are able to inhibit the immune response in relation to that specific antigen, but without global suppression of immunity (ie immune response to other antigens, such as the ovalbumin used here, is not significantly affected).

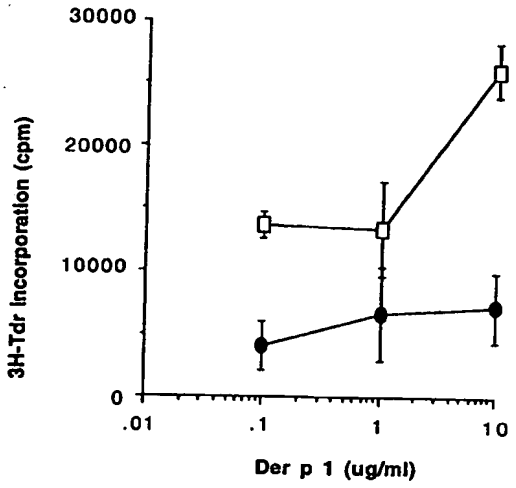


These experiments showed the ability of Notch ligand to induce tolerance in a prophylactic protocol. However, Notch ligand can also induce tolerance to an established immune response as shown in Study 3 below.

Study 3. Notch ligand expressing antigen-presenting cells inhibit established immune responses.

Naïve mice were immunized with 50 μg Der p 1/CFA and 3 weeks later they were injected with p1, 110-131-pulsed DC infected with either *Serrate1* (•) or control (□) virus. Two weeks later mice were reimmunized with 50 μg Der p 1/incomplete Freund's adjuvant and the proliferative response of LN cells to re-stimulation with Der p1 measured as described in Study 1 (A) above.

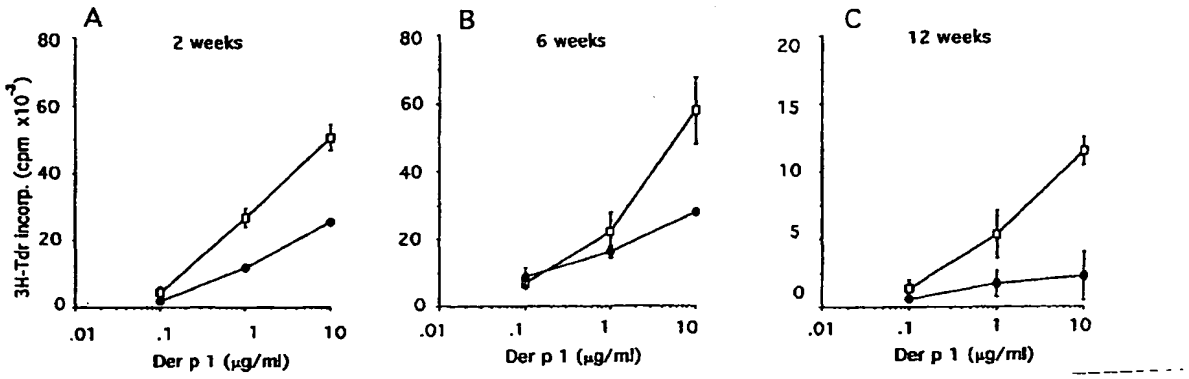
This study shows that Notch ligand can also induce tolerance to an established immune response.



Study 4. Inhibition of T-cell responses induced by Notch ligand expressing antigen-presenting cells is long lived.

p1, 110-131 peptide-pulsed APC infected with either *Serrate1*$^{+}$ (•) or control (□) virus were injected into naïve C57BL/6J mice and (A) 2, (B) 6 or (C) 12 weeks later mice were immunized with Der p 1/CFA and proliferation determined as described in Study 1(A) above.

This study shows that Notch ligand inhibition of T-cell responses is long lived.

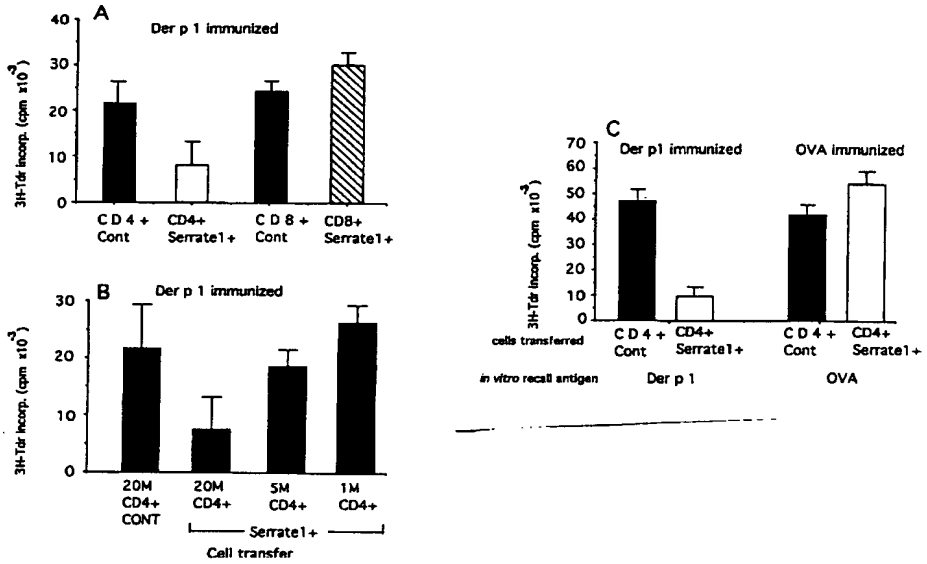


Study 5. Antigen-specific tolerance induced by Notch ligand expressing antigen-presenting cells can be transferred to naïve mice by T-cells.

(A) p1, 110-131 peptide-pulsed APC infected with either *Serrate1* or control virus were injected into naïve C57BL/6J mice and two weeks later CD4+ or CD8+ T cells were isolated from spleens and adoptively transferred to naïve mice at 2 x 10^7/mouse. On the same day mice were immunized with 50 μg Der p 1/CFA and 1 week later LN cells were cultured *in vitro* with Der p 1. Results are presented for proliferation (mean c.p.m ± SD of four mice per group) measured at 72 h in response to re-stimulation with 10 μg/ml Der p1. Transfer of T-cells from mice injected with *Serrate 1*$^+$ (open and shaded bars) or control, (solid and grey bars) DC is shown.

(B) p1, 110-131 peptide-pulsed APC infected with *Serrate1* (grey bars) or control (solid bars) virus were injected into naïve C57BL/6J mice and two weeks later CD4+ - T cells were isolated from spleens and transferred to naïve mice (2x10^7 control CD4+ T cells or 2 x 10^7, 5 x 10^6 or 1 x 10^6 CD4+ T cells from *Serrate 1*$^+$ APC injected mice) which were immunized with 50 μg Der p 1/CFA on the same day. Results are presented for proliferation of LN cells (mean c.p.m. ± SD of four mice per group) measured at 72 h in response to re-stimulation with 10 μg/ml Der p1. (C) p1, 110-131 peptide-pulsed APC infected with *Serrate1* (open bars) or control (solid bars) virus were injected into naïve C57BL/6J mice and 2 weeks later CD4+ T cells were isolated from spleens and 2 x 10^7 cells transferred to naïve mice which were immunized with 50 μg Der p1/CFA or OVA/CFA on the same day. Results are presented for proliferation of LN cells (mean c.p.m. ± SD of four mice per group) measured at 72 h in response to re-stimulation with 10μg/ml Der p1 or 800 μg/ml OVA.

This study shows that antigen-specific tolerance induced by Notch ligand can be transferred to naïve mice by T-cells (infectious tolerance).



3. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further, that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 1/08/02

Jonathan R. Lamb

CURRICULUM VITAE

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Qualifications:

1974	Bachelor of Dental Surgery BDS, Edinburgh University
1976	Bachelor of Arts BA 2nd class, Physiological Sciences (School of Natural Sciences), Oxford University
1980	Doctor of Philosophy PhD, Pathology (Immunology), Faculty of Medicine, London University
1983	Master of Arts MA, Physiological Sciences (School of Natural Sciences), Oxford University
1989	Member of Royal College of Pathologists, MRCPath
1996	Fellow of Royal College of Pathologists, FRCPath
2000	Doctor of Science DSc, Immunology, Faculty of Medicine, Edinburgh University
2002	Fellow of the Royal Society of Edinburgh, FRSE
2002	Fellow Academy of Medical Sciences, FMedSci

University Education:

1970-74	School of Dental Surgery, Faculty of Medicine, Edinburgh University
1974-76	Brasenose College, Oxford University

1977-80 Guy's Hospital Medical and Dental Schools, London University

Honours:

1970-74 General Dental Council Scholar

1971 Distinctions in Physiology and Biochemistry, Merit in Anatomy

1973 Merits in Ethics, Economics and Jurisprudence

1974 Class medals in Forensic Odontology and Periodontology

1983 American Society for Histocompatibility and Immunogenetics Young Investigator Award

1987 Bradley Senior Scholar in Biomedicine, University of Wisconsin, Blood Center of Southeastern Wisconsin, Milwaukee, MI, USA

Appointments:

1976 Dental House Surgeon, Department of Oral Immunology and Microbiology, Guy's Hospital Medical and Dental Schools, London

1977-80 MRC Training Fellow, Department of Oral Immunology and Microbiology, Guy's Hospital Medical and Dental Schools, London

1980-82 Research Assistant Professor, Department of Paediatrics and Immunologic Oncology Division, Lombardi Cancer Research Center, Georgetown University School of Medicine, Washington DC, USA.

1982 Visiting Scientist, Depts. of Immunology & Molecular Genetics, Scripps Clinic and Research Foundation, La Jolla, CA, USA.

1982-85 Scientific Research Fellow, Imperial Cancer Research Fund, Tumour Immunology Unit, Dept. of Zoology & School of Medicine, University College London

1985-90 MRC Scientist, MRC Tuberculosis & Related Infections Unit and Department of Immunology, Royal Postgraduate Medical School, London

1988-90 Honorary Senior Lecturer, Department of Immunology, Royal Postgraduate Medical School, London

1990-97 Professor of Immunology, St. Mary's Hospital Medical School and Department of Biology, Imperial College of Science, Technology and Medicine, London

1994-97 Honorary Consultant in Clinical Immunology, St. Mary's Hospital, Praed Street, London

1997-	Professor of Respiratory Science, MRC Centre for Inflammation Research and Respiratory Medicine Unit, Edinburgh University, Medical School, Edinburgh
1997-	Visiting Professor, Department of Biology, Imperial College of Science, Technology and Medicine, London
2001-	Visiting Professor, Division of Paediatric Surgery, Queen Mary Hospital, University of Hong Kong, HK

Committees and Societies:

1977-	British Society for Immunology
1982-97	Antibody Club
1986-	Editorial Board - Immunology
1988-92	Research Sub-committee of the Arthritis and Rheumatism Council
1990-95	Specialist Advisory Committee in Immunology, University of London
1990-95	St. Mary's Hospital Medical School, Medical Studies Committee
1990-95	St. Mary's Hospital Medical School, Higher Degrees Committee
1991-94	Chairman - Basic Science Committee British Society for Allergy and Clinical Immunology
1992	WHO/UNDP Programme for Vaccine Development - Committee to Define Research Priorities for Future Vaccine Adjuvants
1992-95	WHO Trans-disease Vaccinology Steering Committee
1993-95	St. Mary's Hospital Medical School, Research Sub-committee
1993	WHO/IUIS/IAACI Committee on Recombinant Allergens & Synthetic Epitopes
1993-99	Editorial Board Clinical & Experimental Allergy
1995-99	Editorial Board of Journal of Allergy & Clinical Immunology
1995-96	MRC ROPA Panel Member
1995-98	MRC Clinical Training & Development Panel
1995-2001	Executive Committee of European Science Foundation Network on
HLA &	Allergy

1995	ARC Committee for Clinical & Traveling Fellowships
1996-98	MRC Review of Research Training and Career Development Sub-committee
1995-97	Convener 2nd Year Immunology Course (BSc)
1996-2001	External Examiner in Immunology (BSc Hons), Glasgow University
1997-	Editorial Board International Archives of Allergy and Clinical Immunology
1997-01	National Asthma Campaign Research Committee
1998	Cofounder of Lorantis Limited
1998-2000	Board of Directors of Lorantis Limited
1998-	Scientific Advisory Board of Lorantis Limited
1998-	Associate Editor of Thorax
1998-	MRC Advisory Board (Molecular & Cellular Medicine)
1998-	Faculty of Medicine Postgraduate Studies Committee
1998-	Animal User Committee - Edinburgh University
1999-	MRC Physiological Medicine & Infections Board
1999-	Departmental & Divisional Chairman of Health & Safety Committee - Faculty of Medicine
1999-	Management Board of the MRC Centre of Inflammation Research Edinburgh University
1998-2000	Senior Consultant to ALK Abello A/S, Horsholm, Denmark
1999-	Senior Consultant to Glaxo Smith Kline in Respiratory Medicine
1999-	Scientific Advisory Board of SR Pharma
2000-	Biological Research Resources Management Committee
2000-	Faculty of Medicine Promotions & Honorary Status Committee
2001	New Royal Infirmary Edinburgh Animal Facility Sub-committee
2001	Faculty of Medicine Research Exploitation Committee

2002- External Examiner MSc (Immunology) Nottingham University

2002- Health & Safety Advisory Committee Academic Block New Royal Infirmary Edinburgh

Publications:

1. Lamb, J. R., Kontiainen, S. & Lehner, T. (1979) The generation of specific T cell suppressor function induced by *Streptococcus mutans* in monkeys and mice. *Infect. Immun.* 26: 903-909.

2. Lamb, J. R., Kontiainen, S. & Lehner, T. (1980) A comparative study of the generation of specific T cell helper function induced by *Steptococcus mutans* in monkeys and mice. *J. Immunol.* 124: 2384-2389.

3. Zanders, E. D., Lamb, J. R., Kontiainen, S. & Lehner, T. (1980) Partial characterization of murine and monkey helper factor to a streptococcal antigen. *Immunology*41: 587-596.

4. Lamb, J. R., Zanders, E. D., Kontiainen, S. & Lehner, T. (1980) Immunochemical properties of antigen specific monkey T cell suppressor factor induced with a *Streptococcus mutans* antigen. *Infect. Immun.* 30: 766-772.

5. Lamb, J. R., Zanders, E. D., Kontiainen, S. & Lehner, T. (1981) Regulation and specificity of the immune response to an oral *Streptococcus mutans* antigen by T cell helper and suppressor factors and B cell antibodies. *Arch. Oral Biol.* 26: 745-751.

6. Lamb, J. R., Zanders, E. D., Sanderson, A. R., Ward, P. J., Feldmann, M., Kontiainen, S., Lehner, T. & Woody, J. N. (1981) Antigen specific helper factor reacts with antibodies to human β_2 microglobulin. *J. Immunol.* 127: 231-234.

7. Lehner, T., Lamb, J. R., Welsh, K. L. & Batchelor, J. R. (1981) The association between HLA-DR antigens and high and low avidity helper cell function to a streptococcal antigen in the control of dental caries. *Nature* 292: 770-772.

8. Lehner, T., Lamb, J. R. & Kontiainen, S. (1982) Cell bound helper and suppressor factors in primate lymphocytes. *Clin. Exp. Immunol.*47: 706-714.

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12. Lamb, J. R., Eckels, D. D., Ketterer, E. A., Sell, T. W. & Woody, J. N. (1982) Antigen specific human T lymphocyte clones: mechanisms of inhibition of proliferative responses by xenoantiserum to human non-polymorphic HLA-DR antigens. *J. Immunol.* 129: 1085-1090.

13. Lamb, J. R., Woody, J. N., Hartzman, R. J. & Eckels, D. D. (1982) *In vitro* influenza virus specific antibody production in man: antigen specific and HLA restricted induction of helper activity mediated by cloned human T lymphocytes. *J. Immunol.* 129: 1465-1470.

14. Lamb, J. R., Eckels, D. D., Lake, P., Woody, J. N. & Green, N. (1982) Human T cell clones recognize chemically synthesized peptides of influenza haemagglutinin. *Nature* 300: 66-69.

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17. Lamb, J. R., Skidmore, B. J., Green, N., Chiller, J. & Feldmann, M. (1983) Induction of tolerance in influenza virus immune T lymphocyte clones with synthetic peptides of influenza haemagglutinin. *J. Exp. Med.* 157: 1434-1447.

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21. Lamb, J. R. & Feldmann, M. (1984) Essential requirement for major histocompatibility complex recognition of T cell tolerance induction. *Nature* 308: 72-74.

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